USE OF METHANOTROPHIC BACTERIA IN GAS PHASE BIOREACTORS TO ABATE METHANE IN COAL MINE ATMOSPHERES

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Keywords: Methanotrophic Bacteria, Methane Removal From Mine Atmospheres, Gas Phase Bioreactors

Introduction

Coal mining activities often lead to the release of methane into the mine atmosphere from subterranean pockets that are disturbed during the normal course of mining. This methane can pose a distinct explosion hazard in the mine environment when combined with oxygen from air. It has been reported that the explosive range for methane in air is 5.53% to 14% with methane concentrations above 14% burning without explosion (10). In reality, many mine operations have safety requirements dictating evacuation if mine methane levels exceed 1-2%, since the accidental ignition of methane at concentrations below 5.53% may initiate coal dust explosions (3). Thus, the presence of methane in mines can result in economic loss. This is due to the need to either install ventilation systems and sustain air flow for maintaining methane at safe levels, or terminate operations and evacuate the mine if methane concentrations exceed those deemed safe.

Certain types of bacteria collectively known as methanotrophs are capable of utilizing methane as their sole source of cellular carbon and energy (9). The methanotrophs are nonpathogenic and taxonomically are assigned to several different genera. These bacteria are designated as type I or type II depending on the intracytoplasmic membrane arrangement displayed when grown on methane (1). Methanotrophic bacteria aerobically oxidize methane via a sequential pathway with biomass, carbon dioxide and water being the primary end products of the process (2). Some isolates under certain conditions also have the capability to grow on alternate carbon and energy sources such as alcohols, propane, short chained organic acids, hexadecane, etc. (4).

The methanotrophs are ubiquitous in nature and actively grow in environments where both methane and oxygen or alternate growth substrates are available. This type of environment is most typically found in rich soils, water, and upper layers of

sediments from lakes, harbors, estuaries, ponds, ditches, marshes, and other sites of active methanogenesis (5,8). As a result of their metabolic activities in these environments, methanotrophic bacteria are believed to play a key role in eutrophication by capturing and locking into their ecosystem the carbon from methane (6).

Due to their unique ability to utilize methane as a sole carbon and energy source, methanotrophic bacteria appear to be ideally suited for growth in gas phase bioreactors. In these reactors methane is readily available for cellular metabolism. As such, gas phase bioreactors offer an advantage over liquid phase bioreactors where under certain conditions methane can become limiting due to its relatively low solubility in water.

This paper reports the results from preliminary studies on the growth of a particular type I methanotrophic bacterium, Methylomonas methanica, in gas phase bioreactors. The ability of these bacteria to strip methane from methane-containing atmospheres such as those sometimes found in mine environments was also examined.

Experimental

Culture Maintenance

Methylomonas methanica isolate number O.S.U. 739 was obtained courtesy of the Ohio State University Department of Microbiology culture collection. The culture was maintained in 50 ml aliquots of CM mineral salts medium (7) contained in 125 ml serum bottles sealed with teflon coated rubber stoppers. The bottles were gassed with approximately 30% methane in air and incubated at 37° C on a rotary shaker. Gas levels in the culture vials were monitored using gas chromatographic analysis as described below. Culture bottles were regassed when either the methane or oxygen levels were depleted. Cultures were transferred to fresh medium at least every two weeks to maintain viability.

Gas Phase Bioreactor Design and Maintenance

The bioreactors were constructed from a 3 X 30 inch i.d. glass column sealed at the open end with a rubber stopper (Figure 1). Flexible 5/32 inch o.d. teflon tubing connected the upper end of the column to a stoppered 1 L Erlenmeyer flask that served as a gas volume reservoir. The flask in turn was connected via tubing to the lower end of the column so that a closed recirculation loop was formed. A peristaltic pump which allowed recirculation of gas through the closed system was situated in line between the gas reservoir flask and the lower end of the column. The column interior was filled with polypropylene bio-rings which acted as supports for the growth of the methanotrophs in the gas phase.

The bioreactors were prepared for growth of M. methanica by removing the stopper from the top of the column and pouring

approximately 50 ml of CM mineral salts medium into the upper end of the column. The medium was allowed to trickle over the bio-rings and collect in the bottom of the column. A 50 ml culture of stationary phase M. methanica grown in serum bottles as described above was then poured into the column in a manner similar to that described for the medium. Both the CM minerals salts medium and the inoculum were allowed to remain as a heel in the base of the column to help humidify the bioreactor. Following this, the stopper was tightly reinserted into the upper end of the column and further secured into place by wrapping with parafilm.

The inoculated bioreactor was incubated at 20±2° C for a period of 3 weeks. During this period, methane levels were targeted to approximately 30% methane in air. The gas mixture was constantly recirculated through the column at a rate of 200 ml per minute and gas levels were monitored via gas chromatography. The bioreactors were regassed to the above target levels whenever the methane or oxygen levels fell below 5.0%. Growth of M. methanica was monitored visually via the appearance of the pink pigmented organism on the bio-rings.

Rates of gas depletion were determined by first flushing the bioreactors with air and then gassing the bioreactors with a known mixture of methane in air. The gas mixture was recirculated through the bioreactor at a rate of 200 ml per minute. Gas levels were monitored via gas chromatography.

Analytical Methods

Gas levels (methane, oxygen, and carbon dioxide) in the serum bottle cultures and the bioreactor were analyzed using a Gow-Mac Series 550P gas chromatograph equipped with a thermal conductivity detector and an Alltech CTRI column. The gas chromatograph was connected to a Hewlett Packard model 3390A integrator. Samples consisted of 600 µl gas volumes manually injected into the gas chromatograph which was operated with helium as the carrier gas at a flow rate of 60 ml per minute under isocratic conditions at 30° C.

Results and Discussion

M. methanica was capable of growing to relatively high densities on the polypropylene bio-ring supports contained in the gas phase bioreactors. This growth was apparent visually in the form of highly pigmented pink biomass which adhered to the supports. The ability to directly visualize the growth of M. methanica throughout the bioreactor due to the organism's distinct pink pigmentation was of significant aid in easy, direct, nondestructive evaluation of growth patterns. Visual observation showed the growth to be distributed relatively evenly over the supports throughout the bioreactor with the exception of somewhat

heavier growth on the supports near the gas/liquid interface in the very bottom portion of the bioreactor.

The biomass in the bioreactor was quantitated by simple weighings which showed the average amount of biomass per support to be approximately 0.2 g (wet weight), with the total amount of biomass in the bioreactor being calculated to be 133.4 g (wet weight).

The M. methanica biomass in the bioreactors was assessed relative to its capability to strip methane from air. Figure 2 illustrates the results of experiments to strip a variety of methane levels from air over a 24 hour period. In these experiments the methane/air mixture inside the bioreactor was allowed to continuously recirculate. As can be seen in Figure 2, 35% methane in a total gas volume of 4.5 L was depleted by 90.4% in 24 hours. As would be anticipated, lower methane levels, e.g. 10.6%, were depleted to below the analytical detection limit in less than 24 hours.

In an effort to better simulate the methane levels likely to be encountered in mine environments, the same experiments were repeated using significantly lower starting methane levels measured at more frequent intervals. The results from these experiments are shown in Figure 3 using computed best fit curves. The data indicate that at levels up to 10% methane in air, the removal of methane by M. methanica is linear with the same rates of removal over the entire range under consideration. This is supported by the similar slopes on all three curves.

Under the conditions employed in the experiments illustrated by Figure 3, (i.e. methane < 12%), rates of methane removal for the 133.4 g (wet weight) of biomass contained in the bioreactor were calculated to be 22.9 mg of methane per hour. At higher methane levels such as 30%-45% methane in air, rates of removal were approximately 60% higher averaging 37.7 mg of methane removed per hour.

Further experimentation is necessary to ascertain the reason(s) for this difference in methane removal rates. One possible explanation could be increased transport of the higher concentrations of methane through the biofilm growing on the bio-rings. This increased transport could thus be making methane more available to cells deep within the biofilm and, as a result, greater rates of overall methane degradation could be observed.

Figure 4 illustrates the change in oxygen and carbon dioxide levels in the column during methane removal by the methanotrophic bacteria. Since oxygen serves as a terminal electron acceptor for the methane oxidation pathway, oxygen levels decrease as methane decreases. Similarly as methane is oxidized, the carbon from methane is either incorporated into bacterial biomass or released from the oxidation process as carbon dioxide, thus

explaining the gradual observed increase in carbon dioxide as methane is removed. In the specific example illustrated, with methane levels starting near 11%, a 50% decrease in methane led to an 11% decrease in oxygen. Concurrently, carbon dioxide increased from below the lower limit of detection to approximately 0.8%. These data indicate that methanotrophic bacterial bioreactors would also lower oxygen levels in coal mines. However, the amount of oxygen removed would be modest relative to the amount of methane eliminated, i.e. methane in a mine environment would usually be below 2% whereas oxygen would be approximately 20%.

Conclusions

Conclusions from these preliminary studies are as follows:

- Methanotrophic bacteria such as M. methanica are capable of growing to significant densities in gas phase bioreactors of the types used in this work.
- These organisms remove significant amounts of methane at significant rates from air/methane mixes, and as such may be of practical use in stripping methane from mine atmospheres.
- Additional work needs to be done to optimize reaction rates. This would include a more refined gas phase bioreactor design to (1) increase overall bacterial cell numbers, and (2) maximize bacteria/gas contact. Concurrently, methanotrophic culture optimization needs to be performed. Experiments already being initiated indicate the methane removal rate can be ultimately increased to at least 10 times those reported in this paper.

Acknowledgement

This work was supported under contract no. DE-AC07-76IDO1570 from the U.S. Department of Energy, to the Idaho National Engineering Laboratory/EG&G Idaho, Inc.

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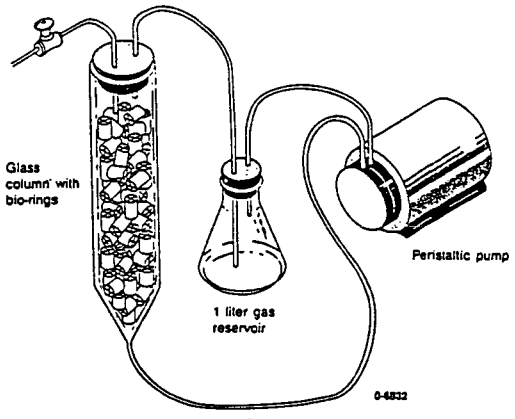

Figure 1
Schematic Diagram of Gas Phase Bioreactor

FIGURE 2
PER CENT METHANE REMOVED IN 24 HOURS
(4.5 L gas volume; 133.4 g (wet weight) M. methanica)

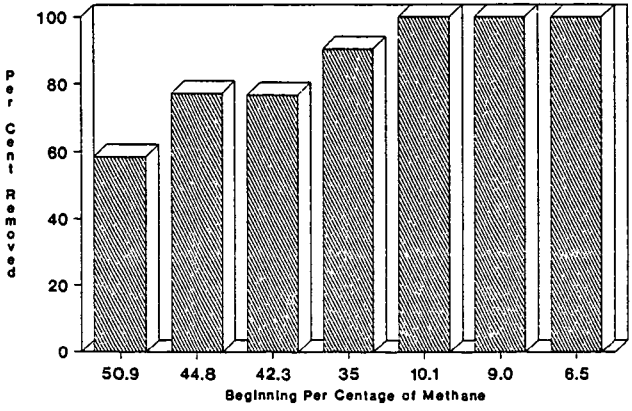

FIGURE 3
METHANE REMOVAL FROM AIR BY M. METHANICA

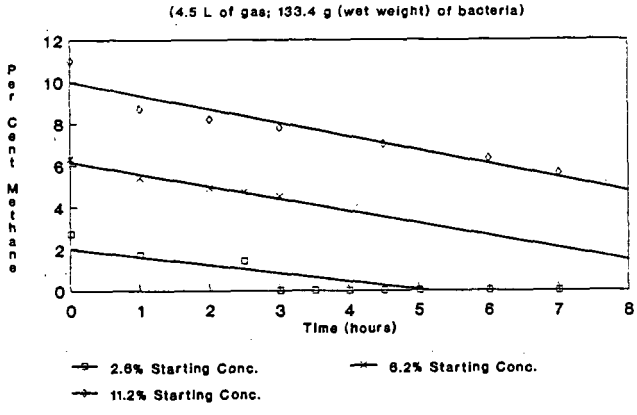

FIGURE 4
CH4 REMOVAL RELATIVE TO O2 AND C02